IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Group Art Unit: UNASSIGNED

JURGEN BODE ET AL.

Examiner: UNASSIGNED

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For: **METHOD FOR MARKER-FREE REPETITIVE DNA EXPRESSION CASSETTE EXCHANGE IN THE GENOME OF CELLS OR PARTS OF CELLS**

Attorney Docket No.: BOET 0130 PUS

PRELIMINARY AMENDMENT

Commissioner for Patents
United States Patent and Trademark Office
Washington, D.C. 20231

Sir:

Please amend the above-identified application as follows:

In The Claims

Please replace claims 1, 3, 5, 7, and 8 as shown below. A marked up version of the amended claims is attached to this Amendment.

1. (Amended) A method for repetitive DNA expression cassette exchange in the genome of cells or parts of cells comprising the steps of

a) integrating into a chromosomal locus of the genome of said cells a first DNA expression cassette carrying a positive-negative selection marker flanked by a wild type FLP-recombinase

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recognition target (FRT) site on one end and a modified heterospecific FRT site on the other end for tagging,

b) selecting cell clones surviving the conditions for positive selection,

c) exchanging said first DNA expression cassette against an incoming second DNA expression cassette located on a circular vector and carrying a homologous or heterologous gene (transgene) of any coding sequence flanked by the same FRT sites as said first DNA expression cassette mediated by the action of FLP-recombinase,

wherein said cells are vertebrate cells which can regenerate to complete organisms, and said parts of cells are nuclei of vertebrate cells, which can be inserted into regenerative cells, and wherein

d) maintaining the conditions for positive selection during cultivation of said cells obtained in step b) while exchanging said first DNA expression cassette against said incoming second DNA expression cassette.

e) using in step c) an incoming second DNA expression cassette which is marker-free, and

f) selecting cell clones obtained after step c) surviving the conditions for negative selection.

3. (Amended) The method according to claim 1 wherein said modified heterospecific FRT site is a FRT spacer mutant.

5. (Amended) The method of claim 1 wherein said vertebrate cells which can be regenerated are vertebrate embryonic stem (ES) cells.

7. (Amended) Regenerative vertebrate cells comprising a modified genome obtainable by the method of claim 1.

8. (Amended) Nuclei of vertebrate cells comprising a modified genome obtainable by the method of claim 1.

10. (Amended) A method for generation of transgenic vertebrates characterized by injecting regenerative vertebrate cells according to claim 7 into blastocysts of said vertebrate.

REMARKS

The claim amendments eliminate multiple dependent and multiple/multiple dependent claims, but otherwise leave the substance of the claims unchanged. Early favorable consideration is respectfully requested.

Respectfully submitted,

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By _____
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Attachment

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In The Claims

1. (Amended) A method for repetitive DNA expression cassette exchange in the genome of cells or parts of cells comprising the steps of

a) integrating into a chromosomal locus of the genome of said cells a first DNA expression cassette carrying a positive-negative selection marker flanked by a wild type FLP-recombinase recognition target (FRT) site on one end and a modified heterospecific FRT site on the other end for tagging,

b) selecting cell clones surviving the conditions for positive selection,

c) exchanging said first DNA expression cassette against an incoming second DNA expression cassette located on a circular vector and carrying a homologous or heterologous gene (transgene) of any coding sequence flanked by the same FRT sites as said first DNA expression cassette mediated by the action of FLP-recombinase,

[characterized in that] wherein said cells are vertebrate cells which can regenerate to complete organisms, and said parts of cells are nuclei of vertebrate cells, which can be inserted into regenerative cells, and [further characterized by] wherein

d) maintaining the conditions for positive selection during cultivation of said cells obtained in step b) while exchanging said first DNA expression cassette against said incoming second DNA expression cassette.

e) using in step c) an incoming second DNA expression cassette which is marker-free, and

f) selecting cell clones obtained after step c) surviving the conditions for negative selection.

3. (Amended) The method according to claim 1 [or 2] wherein said modified heterospecific FRT site is a FRT spacer mutant.

5. (Amended) The method [according to any of the preceding claims] of claim 1 wherein said [regenerative] vertebrate cells which can be regenerated are vertebrate embryonic stem (ES) cells.

7. (Amended) Regenerative vertebrate cells comprising a modified genome obtainable by [a method according to any one of claims 1 to 6] the method of claim 1.

8. (Amended) Nuclei of vertebrate cells comprising a modified genome obtainable by [a method according to any one of claims 1 to 6] the method of claim 1.

10. (Amended) A method for generation of transgenic vertebrates characterized by injecting regenerative vertebrate cells according to claim 7 [or 9] into blastocysts of said vertebrate.